Microanatomy of Myentric Plexus in Large intestine of Human Aborted Foetuses

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INTRODUCTION

The large intestine is a derivative of the midgut and hindgut. It is extending from the caecum to the anal canal. the mucosa is derived from the endoderm and the muscularis externa from the mesoderm. The innervation of these muscles has a major role in the motility of the large intestine. The enteric nervous system contributes to the innervation of the gut. It is considered to be the second brain in the gut [1]. It is a part of the peripheral nervous system. The Enteric Nervous System works autonomously without inputs from the Brain or spinal cord. The neuronal cells present in the enteric nervous system play a critical role in mediating motility reflexes of the gut, its secretions and blood supply. The enteric nervous system is similar to the central nervous system in that it can work autonomously in certain regions of the gastrointestinal tract due to its complexity in nature [2].

The Enteric Nervous System is derived from the neural crest cells. The neural crest cell migrates into the gastrointestinal tract, proliferates, forms plexus and differentiates into neurons and glial cells [3]. The neural crest cells independently innervate the gut. The gut innervation is derived from the vagal and the sacral neural crest cells. The vagal crest cells were highly invasive in nature [3]. It is derived from somite level of 1-7 and from 28 onwards [4].

The incorporating neuroblast cells are the prime source of intra-mural plexus in the gut and is in the craniocaudal direction [4,5,6]. It was accomplished and reached the distal end of the rectum at 12 weeks of gestation. This process was stopped or ceased at any stage, on or before 12 weeks of gestation, leading to the disappearance of the intra-mural plexus at any segment of the gastrointestinal tract [7].

The myenteric ganglions were diverse with respect to size, shape, structure and number of neurons present in them. The available intermuscular space is also one of the contributing factors to the different shapes, sizes and number of ganglia. The myenteric ganglia are usually flat, irregular and angulated outlined borders in longitudinal sections [8].

In the early weeks of Gestation, the enteric neurons are present in the scattered form and they appeared in the ganglionated form as the gestational week advances. these were oval in shape in the serosa of the large intestine in the early weeks of gestation and become elongated in the later stages of gestation. these are oval, elongated and irregular in the muscular and submucosa layers of the large intestine. [9].

The morphological and neurochemical properties of the human Enteric Nervous System are different from other animal species, even during its development, therefore an in-depth investigation of the human Enteric nervous system is necessary [3,10]. The number of ganglia and ganglion cells in

the enteric nervous system is not the same and varies in different locations. The location of biopsy site also important for quantitative analysis of nerve plexus [11].

Morphometric measurements of enteric nervous system components were very useful as a diagnostic tool to identify the disorders related to innervation of gut [12]. The literature reveals that many studies related to myenteric plexus were descriptive approaches and only a few studies were concerned with a quantitative approach [13,14,15].

Many studies were reported earlier about the presence, absence and maturations pattern of myenteric plexus as samples taken as a bit from the oesophagus, a bit from the small intestine and a bit from the large intestine rather than the whole segment.

A valid standard method for quantitative analysis of myenteric plexus has long been debated among enteric neuropathologists and it is essential for better characterization of patients with gut dysmotility[11]. Hence, It is necessary to be aware of the normal quantitative values of the myenteric plexus of any segment of the large intestine of the gastrointestinal tract.

AIMS AND OBJECTIVES

Aim:

To determine the Histomorphometric analysis of the myenteric plexus in the entire large intestine of aborted human foetuses.

Objectives:

1. To study the morphometric profile like Length of the ganglion, Feret diameter of the ganglion, Area occupied by the ganglion, Numerical (Neuronal) density, Total Muscular Area, Myenteric fraction and Inter ganglion distance of the myenteric plexus from the caecum to the anal canal of the large intestine of normal human aborted foetuses.
2. To Correlate these values with weeks of Gestation.

Hypothesis/Research questions:

Will the Histomorphometric analysis of all the segments of the human aborted fetuses of the large intestine be the same?

REVIEW OF LITERATURE

Brief History:

The ganglionated plexus present between the smooth muscle layer of the gut will contribute to intrinsic innervation of the gastrointestinal tract. The quest related to precursor cells and their development are trying to answer for the past 40 years.

In recent days, the novice and advanced techniques pertaining to the molecular mechanism and their pathways made a route for demonstrating these ganglionated plexuses.

In 1954, Yntema and Hammond demonstrated that most enteric neurons are derived from the rhombencephalon (vagal) neural crest cells. Almost 20 years later, in the year 1973, Le Douarin and Teillet mapped the precise location of the enteric nervous system with in the neural crest cells. It was facilitated by the development of the embryonic cell marking technique by Le Douarin in the 1960s. The sacral neural crest cells present more caudal to neuraxis which were also contributed to the enteric nervous system. This was described by Le Douarin and Teillet. Gershon (1990), Pomeranz et al (1991), and

Serbedzija et al (1991) supported this theorem but Allan and Newgreen (1980) and Meijers et al (1989) were opposed.

Okamoto et al (1967) reported that the prime source of myenteric plexus development from neuroblast cells which run in the craniocaudal direction and complete the migration of the entire gut at the age of 12 weeks gestation. The failure of these steps at any moment or stage leads to aganglionosis. The rectosigmoid junction was the distal distant part to be covered by neuroblast cells.

Bandyopadhya R et al (2011) found that all foetuse ganglion cells in the myenteric plexus were present as early as 12 weeks. The well-developed ganglia appeared in the pylorus at 12 weeks, the colon at 14 weeks, and the ileum at 15 weeks of gestation.

Kim HK (2010) et al did a study to obtain comprehensive data on the structural component of the enteric nervous system. They tried to explore the possible diagnostic criteria to evaluate frozen serial sections for intestinal neuronal dysplasia (IND) type B and hypo ganglinosis. Their findings suggested that the morphometric measured factors are useful as diagnostic criteria for intestinal neuronal dysplasia (IND) type B and hypo ganglinosis.

Mandic.P et al (2016) aimed to establish neuronal density per cm^2 surface of the myenteric plexus of all main parts of the digestive tract.

They tried to determine the quantitative differences of these intramural plexus among various parts of the alimentary tract. The lowest value was found in the oesophagus and the highest value was found in the Duodenum. There were noticeable very large differences in the number of neurons found in the different parts of the alimentary tract.

Mandic.P et al (2016) claimed that there were no study reports in the available literature pertaining to the number of neurons in the human colon. The number of neurons in the human transverse colon was 37,836±4,126 neurons/cm^2 and the rectum was 50,106±3,004 neurons/cm^2. The average number of neurons per cm^2 was high in the rectum and stomach when compared to other parts of the digestive tract.

Boschetti E et al (2019) correlate enteric neuron density with clinical features of severe gut dysmotility in the jejunum. The number of neurons per ganglion in the myenteric plexus was 2.2 folds less in severe gut dysmotility patients when compared with normal subjects.

MATERIAL AND METHODS

2.1 Foetal Age Determination:

The foetal age was determined by the parameters like Crown-rump length, foot length, and bi-parietal diameter with relevance to Ultrasonography history.

2.2 Sample Collection:

the samples were obtained within 1-2 hours after delivery.

A para median incision in the abdomen was given and the entire large intestine was exposed. The sample was immersed in 4% Buffered paraformaldehyde for a proper fixation process. The specimen was preserved at 4°C to minimize the post-mortem changes.

2.3 Sample size:

The total sample size was 50 aborted human fetuses. The gestational age was 13 weeks and above.

2.4 Segments of the sample:

The entire large intestine was divided into seven segments. A1 segment from Caecum, A2 segment from Ascending Colon, A3 segment from the Transverse Colon, A4 segment from the Descending colon, A5 segment from the Sigmoid colon, A6 segment from the Rectum and A7 segment from the Anal canal.

2.5 Grouping of Sample:

The foetuses were assigned into two groups. Group A less than ≤ 20 WG and Group B more than ≥ 20 WG.

2.6 Enzyme Histo Chemistry Method:

NADPH diaphorase histochemistry method. An enzyme Histochemistry technique was used to study the tissue preparations.

2.7 Fixation of the sample:

the colonic tissue was fixed in fresh 4% buffered paraformaldehyde for 2 hours at 4°C. they were washed thoroughly after fixation in chilled 0.1M phosphate buffer.

2.8 Cryo protection of the Sample:

The sample was kept at 15% and 30% sucrose solution for 3 hours and 8 hours at 4° C for cryo protection.

2.9 Preparations of sections of the Sample:

The samples were then frozen in an optimum cutting temperature compound. 20 µm thick sections were cut by using a Leica cryostat. The frozen sections were placed on gelatine-coated slides. The slides were stored at -20°C for the further process of enzyme histo chemistry reaction. The stored sections on the glass slide were washed several times with 0.1 M phosphate buffer before starting the NADPH Diaphorase action.

2.10 Preparation of Gelatin Coated slides:

Prepare the Gelatin coating solution by dissolving 5 grams of Gelatin in 1 litre of heated deionized water. The temperature should not exceed 45°C. after the gelatin has dissolved, add 0.5 grams of Chromium Potassium Sulfate Dodecahydrate. ($CrK(So_4)_2$ .12 H_2O.)

2.11 Enzyme Activity:

NADPH diaphorase activity was visible by incubating the sections in 10 ml of 0.1 M tris-Cl buffer containing 10mg NADPH,1 mg of Nitro blue tetrazolium NBT and 0.3% triton X100 at 37° C for 45 minutes to 1 hour in the incubator in dark. The reaction was monitored under a dissecting microscope and was terminated by washing the tissues gently with chilled 0.1 M phosphate buffer when the stain was sufficiently intense. The sections were mounted in a mixture of glycerol and phosphate buffer.

2.12 Capture the images:

The stained sections were examined under a microscope and images were captured using camera connected binocular microscope. The images were saved in JPEG format with minimum compression and maximum quality. The images were analysed by using Image J software. Before making measurements, the system was calibrated using a micrometre scale for the magnification at which the images were acquired.

INCLUSION AND EXCLUSION CRITERIA:

- All the mothers who had no medical illness during the pregnancy and none of the foetuses had any congenital anomalies were included.
- The foetuses with neural tube defects were excluded from the study sample.

STATISTICAL ANALYSIS:

Statistical significance was determined using SPSS ver.20.0[IBM Corp., Armonk, N.Y., USA]. The data were expressed as mean± standard deviation. The independent sample 't-test was done for parametric analysis. P- value of less than 0.05 was regarded as statistically significant**.**

Fig 1 : Foetal Age Determination by measuring Crown Rump Length.

Fig 2: Para median incision & exposing the Large intestine of the Sample.

Fig 3: Preparation for segmental division of large Intestine.

Fig 4: OCT medium tissue Blocks for Cryostat microtome.

RESULTS

5.1 Maternal Age Distribution:

Most of the sample's maternal age was between 25 to 30 years old.

5.2 Blood Group Distribution:

Most of the sample's maternal blood group was the 'O' group.

5.3 Gestational Week Distribution:

Most of the samples obtained belong to the second trimester & early third trimester.

5.4 Gravid Distribution:

The maximum number of samples belongs to Second Gravida.

5.5 Gender Distribution:

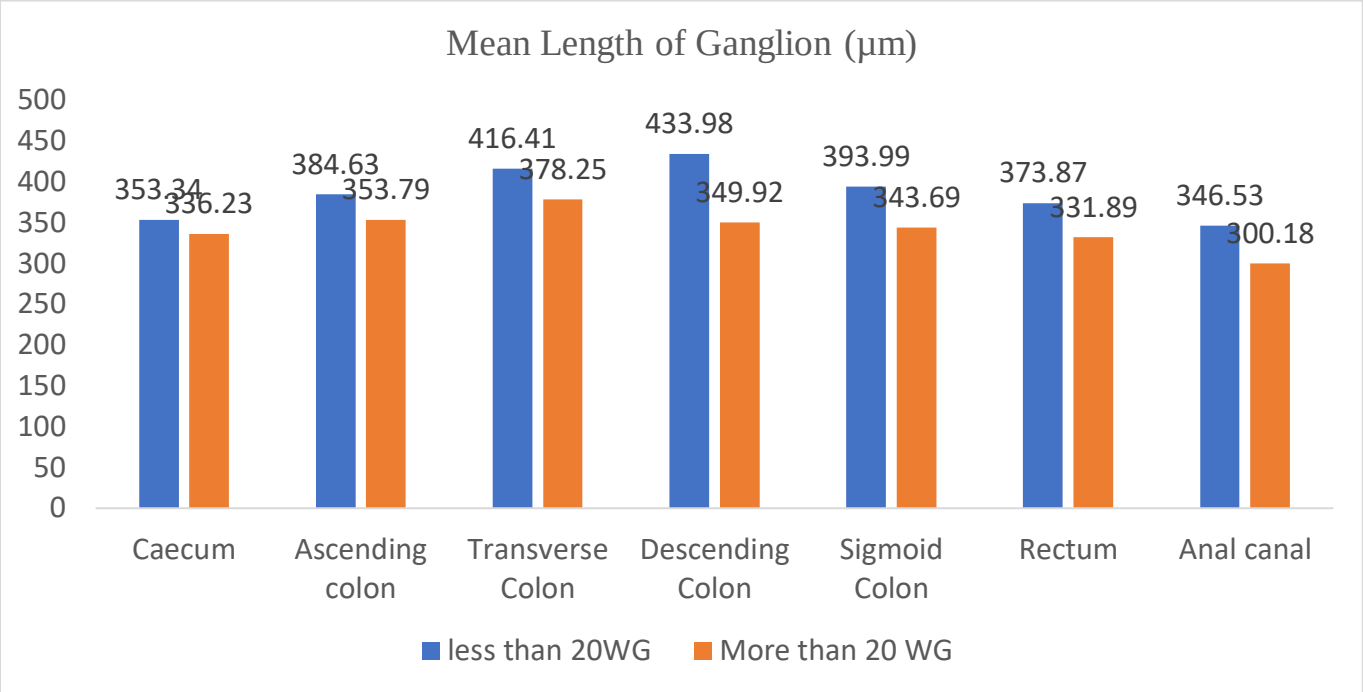
Most of the samples were belongs to Male Gender.

5.6 Mean Length of Ganglion (µm) in Group A and Group B.

S.No.	Caecum	Ascending Colon	Transverse Colon	Descending Colon	Sigmoid Colon	Rectum	Anal canal
Group A ≤ 20 Wk	351.06 ± 79.61	379.91 ± 116.55	397.23 ± 123.13	419.93 ± 115.77	394.35 ± 75.86	373.80 ± 73.60	346.57 ± 63.49
Group B ≥20 Wk	331.42 ± 47.32	345.65 ± 80.33	362.03 ± 109.29	350.90 ± 85.16	337.48 ± 73.51	330.58 ± 65.23	293.41 ± 59.83
P value	0.108	0. 220	0.699	0.204	0.951	0.426	0.687

Table 1 showing the mean length of the ganglion in different segments of the large intestine. The comparative values in less than 20 WG and more than 20 WG.

- The mean length of Ganglion values was more in less than 20 weeks gestation when compared to more than 20 weeks gestation period.
- The mean length of the ganglion was greater in the descending colon, followed by the transverse and sigmoid colon. The least length was shown in the anal canal of less than 20 weeks of gestation period.
- The mean length of the ganglion in more than 20 weeks of gestation was greater in the transverse colon followed by descending and ascending colon. the p values were not significant.

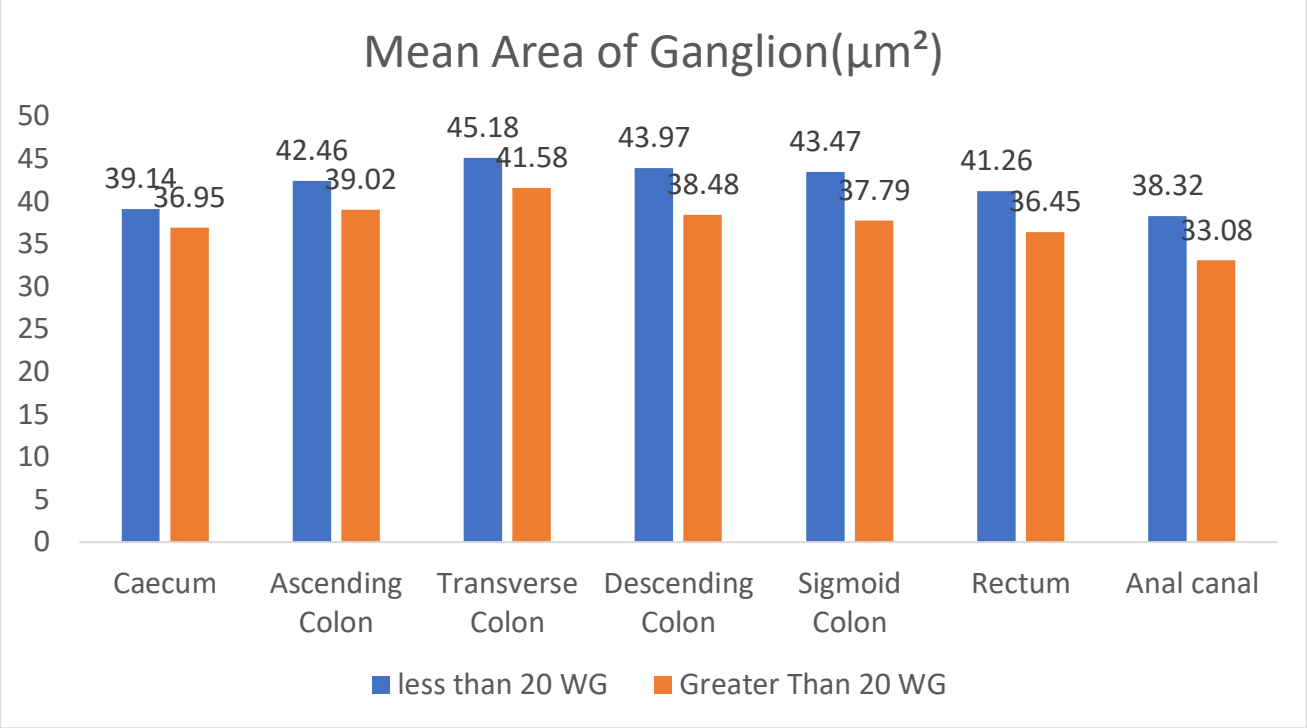

Mean Length of Ganglion (µm)
500
450
400
350
300
250
200
150
100
50
0
353.34
336.23
384.63
353.79
416.41
378.25
433.98
349.92
393.99
343.69
373.87
331.89
346.53
300.18
Caecum
Ascending colon
Transverse Colon
Descending Colon
Sigmoid Colon
Rectum
Anal canal
less than 20WG
More than 20 WG

5.7 Area of Ganglion(μm^2) in Group A and Group B

S.No.	Caecum	Ascending Colon	Transverse Colon	Descending Colon	Sigmoid Colon	Rectum	Anal canal
Group A ≤ 20 Wk	40.789± 10.36	43.579 ± 13.26	46.070 ± 12.11	55.035 ± 9.12	45.864 ± 9.22	44.302 ± 9.98	38.413 ± 7.39
Group B ≥20 Wk	37.270 ± 4.54	38.53 ± 8.64	41.377 ± 11.56	38.761 ± 8.98	36.882 ± 8.03	36.107 ± 6.76	31.92 ± 6.74
P value	0.001[x]	0.101	0.888	0.058[*]	0.633	0.064	0.669

Table 2 showing the meanArea of the ganglion in different segments of the large intestine. The comparative values in less than 20 WG and more than 20 WG.

- The area occupied by the myenteric plexus was greater in the descending colon followed by the transverse and sigmoid colon and it was least in the anal canal of less than 20 weeks gestation group.
- The area was greater in the transverse colon followed by descending and ascending colon. It was the least in the anal canal.
- The p-value in the caecum was 0.001, and in the descending colon 0.058, which are significant.

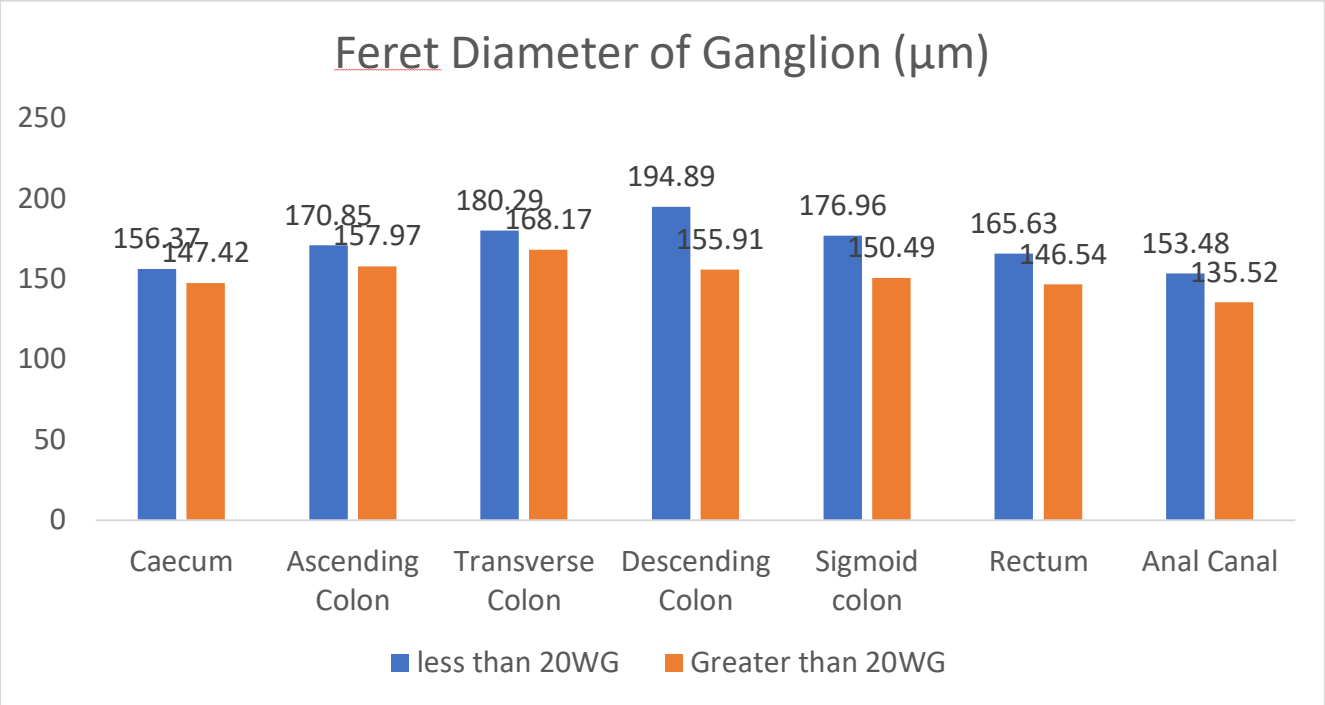

Mean Area of Ganglion(µm²)
50
45
40
35
30
25
20
15
10
5
0
39.14
36.95
42.46
39.02
45.18
41.58
43.97
38.48
43.47
37.79
41.26
36.45
38.32
33.08
Caecum
Ascending Colon
Transverse Colon
Descending Colon
Sigmoid Colon
Rectum
Anal canal
less than 20 WG
Greater Than 20 WG

5.8 Feret diameter of the ganglion (µm) in Group A and Group B:

S.No.	Caecum	Ascending Colon	Transverse Colon	Descending Colon	Sigmoid Colon	Rectum	Anal canal
Group A ≤ 20 Wk	154.18 ± 36.21	160.06 ± 54.86	170.41 ± 51.27	184.94 ± 52.86	173.45 ± 33.50	162.32 ± 35.34	146.39 ± 30.00
Group B ≥20 Wk	147.61 ± 23.81	154.92 ± 38.65	161.73 ± 49.28	153.27 ± 42.06	146.59 ± 34.83	145.09 ± 31.12	131.90 ± 27.94
P value	0.138	0.227	0.695	0.315	0. 631	0.560	0.406

Table 3 showing the mean ferret diameter of the ganglion in different segments of the large intestine. The comparative values in less than 20 WG and more than 20 WG.

- The feret diameter was more in the descending colon followed by the sigmoid and transverse colon and it was lowest in the anal canal of less than 20 weeks gestation period.
- The feret diameter was greater in the transverse colon followed by ascending colon and descending colon. It was least in the anal canal in more than 20 weeks of the gestation period.

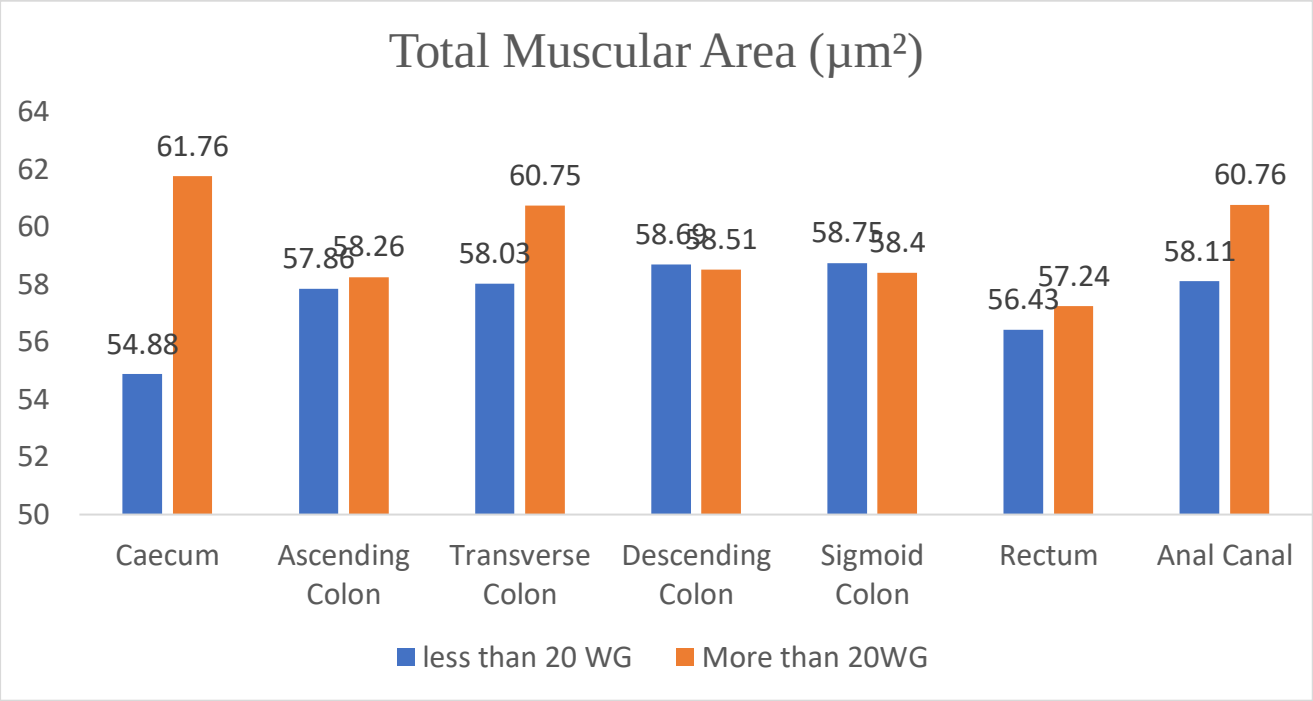

Feret Diameter of Ganglion (µm)
250
200
150
100
50
0
156.37
147.42
170.85
157.97
180.29
168.17
194.89
155.91
176.96
150.49
165.63
146.54
153.48
135.52
Caecum
Ascending Colon
Transverse Colon
Descending Colon
Sigmoid colon
Rectum
Anal Canal
less than 20WG
Greater than 20WG

5.9 Total Muscular Area(μm^2) of Large Intestine in Group A and Group B:

S.No.	Caecum	Ascending Colon	Transverse Colon	Descending Colon	Sigmoid Colon	Rectum	Anal canal
Group A ≤ 20 Wk	55.93 ± 9.07	58.31 ± 11.34	60.50 ± 13.57	71.46 ± 14.28	61.00 ± 9.68	59.16± 10.24	58.36 ± 13.17
Group B ≥20 Wk	60.66 ± 10.09	57.85± 12.95	58.72± 16.56	57.43 ± 12.70	56.12± 12.78	56.42± 11.84	53.13± 12.00
P value	0.512	0.264	0.234	0.156	0.040*	0.318	0.256

Table 4 showing the mean total muscular area of gut wall in different segments of the large intestine. The comparative values in less than 20 WG and more than 20 WG.

- The total muscular value was greater in the descending colon followed by the sigmoid colon and transverse colon. it was least in the caecum part in the less than 20 weeks of gestation period.
- The total muscular area is greater in the caecum followed by the transverse colon and ascending colon. it was least in the anal canal in more than 20 weeks gestation period.
- The p value is significant in the sigmoid colon.

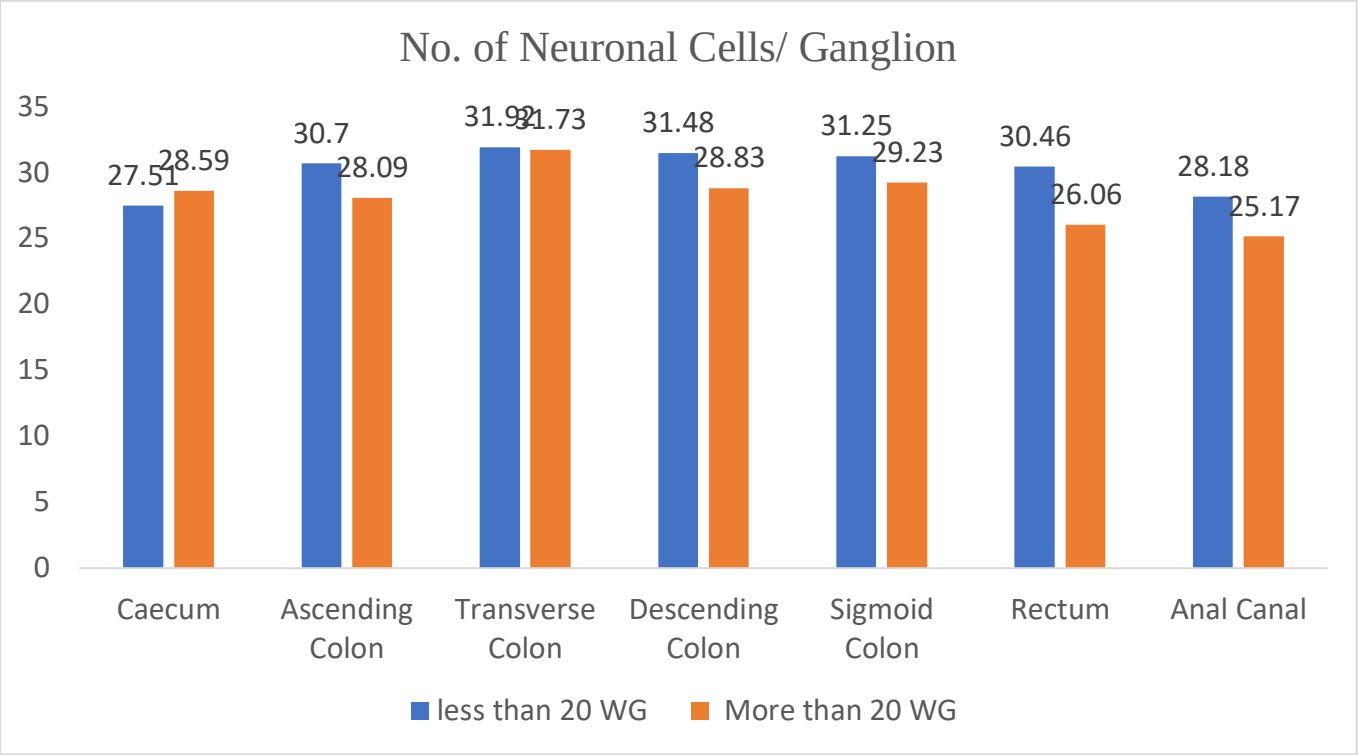

Total Muscular Area (µm²)
64
62
60
58
56
54
52
50
61.76
54.88
57.86
58.26
58.03
60.75
58.69
58.51
58.75
58.4
56.43
57.24
58.11
60.76
Caecum
Ascending Colon
Transverse Colon
Descending Colon
Sigmoid Colon
Rectum
Anal Canal
less than 20 WG
More than 20WG

5.10 No. of Neuron Cells per Ganglion in Group A and Group B:

S.No.	Caecum	Ascending Colon	Transverse Colon	Descending Colon	Sigmoid Colon	Rectum	Anal canal
Group A ≤ 20 Wk	27.67 ± 6.79	30.00 ± 13.46	33.19 ± 9.98	31.98 ± 7.52	32.13 ± 8.73	30.77± 9.62	26.76 ± 7.24
Group B ≥20 Wk	28.51 ± 6.69	28.05± 7.77	30.76± 9.83	28.30± 6.64	28.96± 7.85	26.04± 7.60	24.15± 5.17
P value	0.480	0.474	0.998	0.536	0.945	0.275	0.042*

Table 5 showing the Mean Number of Neuron Cells per Ganglion in different segments of the large intestine. The comparative values in less than 20 WG and more than 20 WG.

- The number of ganglions was high in the transverse colon followed by the sigmoid colon and descending colon. These values were lower in the anal canal in the less than 20 weeks gestation period group.
- The number of ganglions was more in the transverse colon followed by the sigmoid colon and caecum and it was less in the anal canal in the more than 20 weeks gestation period group.
- The p-value significant in the anal canal.

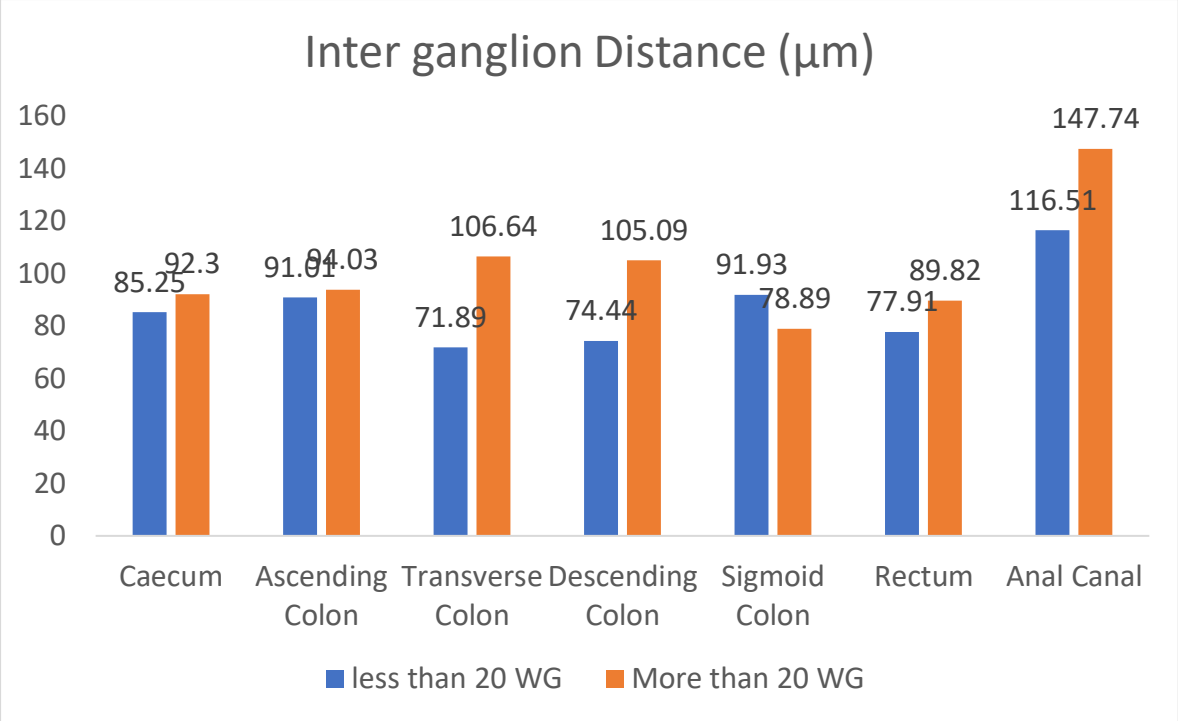

No. of Neuronal Cells/ Ganglion
35
30
25
20
15
10
5
0
27.51
28.59
30.7
28.09
31.92
31.73
31.48
28.83
31.25
29.23
30.46
26.06
28.18
25.17
Caecum
Ascending Colon
Transverse Colon
Descending Colon
Sigmoid Colon
Rectum
Anal Canal
less than 20 WG
More than 20 WG

5.11 Inter Ganglion Distance (µm) in Group A and Group B:

S.No.	Caecum	Ascending Colon	Transverse Colon	Descending Colon	Sigmoid Colon	Rectum	Anal canal
Group A ≤ 20 Wk	85.58 ± 29.20	87.71 ± 32.64	87.15 ± 10.40	96.62 ± 8.98	93.27 ± 31.83	78.86 ± 38.09	126.94 ± 23.87
Group B ≥20 Wk	69.38 ± 9.96	93.85 ± 18.90	66.41 ± 8.05	70.03 ± 19.97	70.31 ± 20.10	83.20 ± 26.73	107.67 ± 30.17
P value	0.094	0.113	0.206	0.423	0.155	0.003^{x}	0.865

Table 6 shows the Mean Inter Ganglion Distance between the Ganglion in the myenteric plexus in different segments of the large intestine. The comparative values in less than 20 WG and more than 20

- Tthe longest inter ganglion distance was seen in the anal canal followed by descending colon and sigmoid colon and the shortest was seen in the rectum part digestive system of less than 20 weeks gestation period.
- The longest inter ganglion distance was in the anal canal followed by ascending colon and rectum in more than 20 weeks of the gestation period. The shortest was seen in the transverse colon.
- The p-value is significant in the rectum.

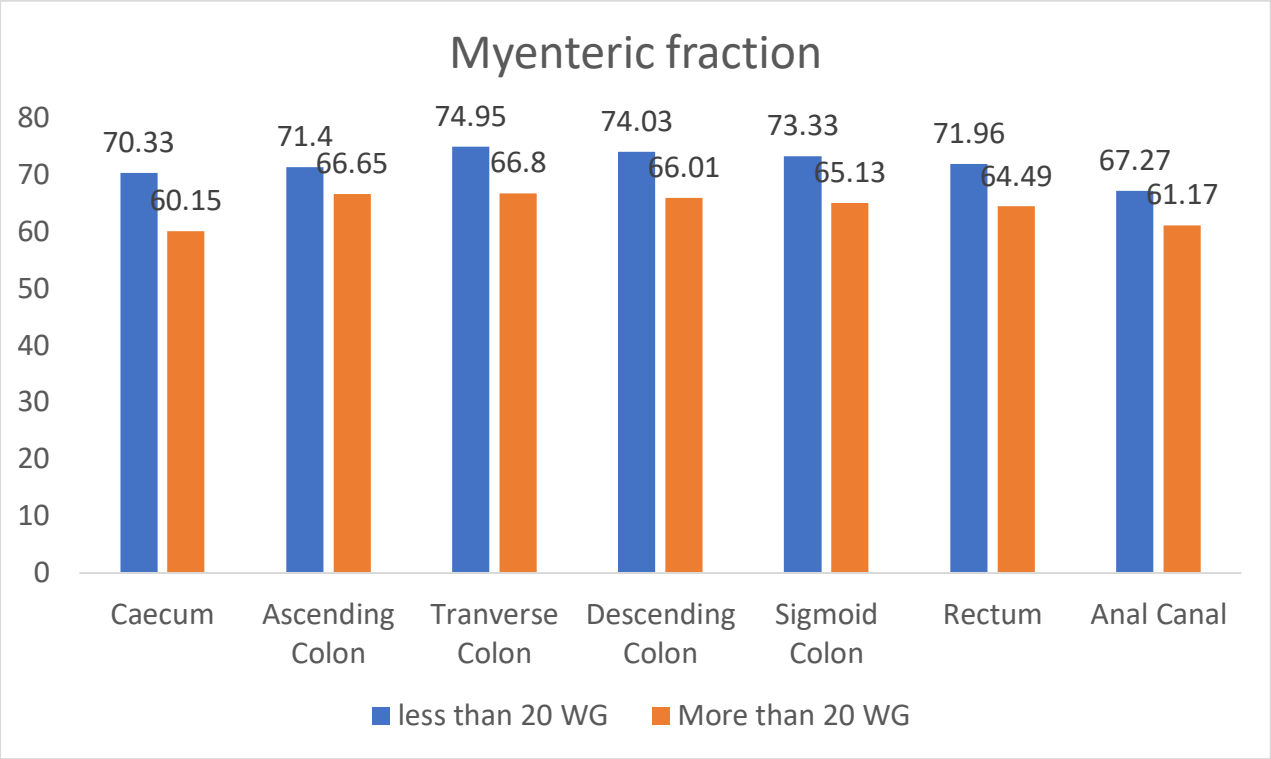

Inter ganglion Distance (µm)
160
140
120
100
80
60
40
20
0
85.25
92.3
91.01
94.03
71.89
106.64
74.44
105.09
91.93
78.89
77.91
89.82
116.51
147.74
Caecum
Ascending Colon
Transverse Colon
Descending Colon
Sigmoid Colon
Rectum
Anal Canal
less than 20 WG
More than 20 WG

5.12 Myenteric Fraction (Ratio) In Group A And Group B:

S.No.	Caecum	Ascending Colon	Transverse Colon	Descending Colon	Sigmoid Colon	Rectum	Anal canal
Group A ≤ 20 Wk	69.38 ± 9.96	69.65 ± 9.88	72.25 ± 8.33	72.46 ± 7.99	71.96 ± 7.62	71.11 ± 7.64	64.08 ± 9.18
Group B ≥20 Wk	61.67 ± 8.85	65.70 ± 8.58	66.34 ± 8.85	66.63 ± 10.15	65.48 ± 8.76	64.99 ± 10.29	61.43 ± 10.07
P value	0.671	0.676	0.948	0.449	0.826	0.286	0.459

Table 7 shows the Myenteric fraction in different segments of the large intestine. The comparative values in less than 20 WG and more than 20 WG. It is the ratio between the No of Neuron cells and the Total Muscular area.

- The myenteric fraction was greater in the descending colon followed by the transverse colon and in the sigmoid colon in the less than 20 weeks gestation period group. It was least in the anal canal in the less than 20 weeks gestation period group.
- The myenteric fraction was greater in the descending colon followed by the transverse colon and sigmoid colon in the more than 20 weeks gestation period group. It was least in the anal canal.

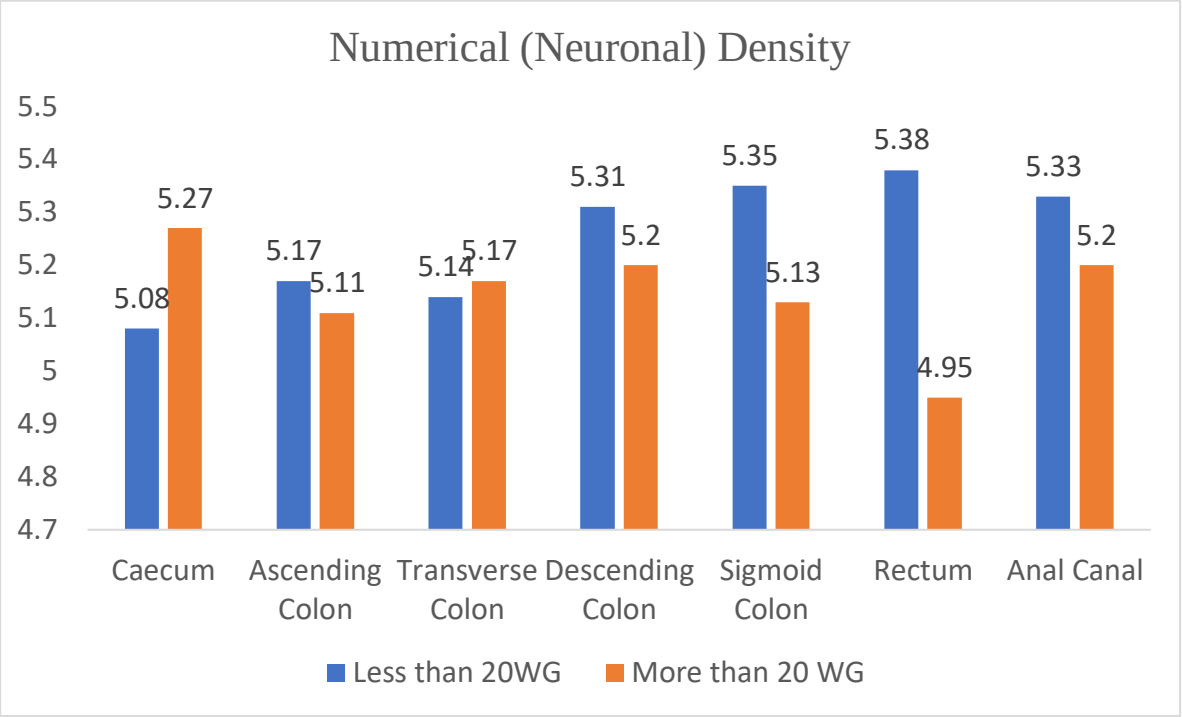

Myenteric fraction
80
70
60
50
40
30
20
10
0
70.33
60.15
71.4
66.65
74.95
66.8
74.03
66.01
73.33
65.13
71.96
64.49
67.27
61.17
Caecum
Ascending Colon
Tranverse Colon
Descending Colon
Sigmoid Colon
Rectum
Anal Canal
less than 20 WG
More than 20 WG

5.13 Neuronal Density in Group A and Group B:

S.No.	Caecum	Ascending Colon	Transverse Colon	Descending Colon	Sigmoid Colon	Rectum	Anal canal
Group A ≤ 20 Wk	5.06 ± 0.67	8.03 ± 0.51	5.16 ± 0.55	5.32 ± 0.67	5.29 ± 0.85	5.41± 0.79	5.12 ± 0.83
Group B ≥20 Wk	5.26 ± 0.77	5.06± 0.62	5.15± 0.72	5.10± 0.77	5.07± 0.76	4.83± 0.73	5.14± 0.62
P value	0.340	0.126	0.399	0.539	0.921	0.955	0.127

Table 7 shows the Neuronal Density in different segments of the large intestine. The comparative values in less than 20 WG and more than 20 WG. It is the minimum number of neurons present per unit distance of segments of large Intestine.

- The Neuronal Density in different Segments are approximately 5 neurons in number per unit distance spread. These values were closely in the Group A and Group B. it shows Uniform distribution among the segments of Large intestine.


Numerical (Neuronal) Density
5.5
5.4
5.3
5.2
5.1
5
4.9
4.8
4.7
5.08
5.27
5.17
5.11
5.14
5.17
5.31
5.2
5.35
5.13
5.38
4.95
5.33
5.2
Caecum
Ascending Colon
Transverse Colon
Descending Colon
Sigmoid Colon
Rectum
Anal Canal
Less than 20WG
More than 20 WG

5.14 Microscopic Images (NADPH Diaphorase stained images)

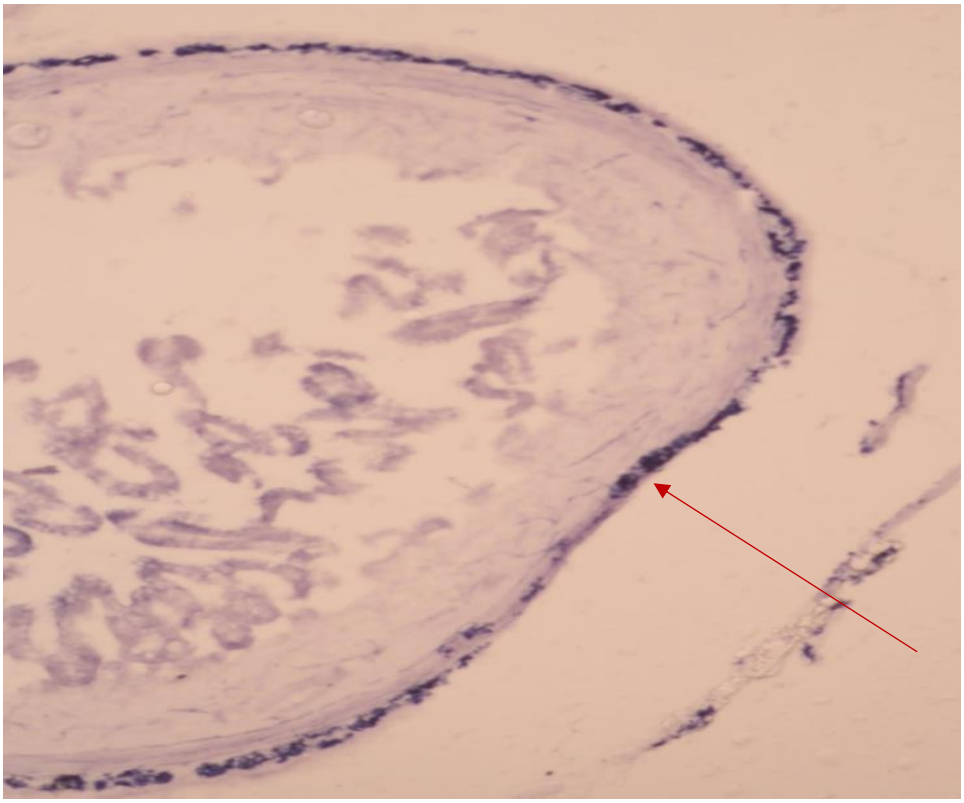


Fig 5: Shows the myenteric plexus present between the outer longitudinal and inner circular muscle layer.

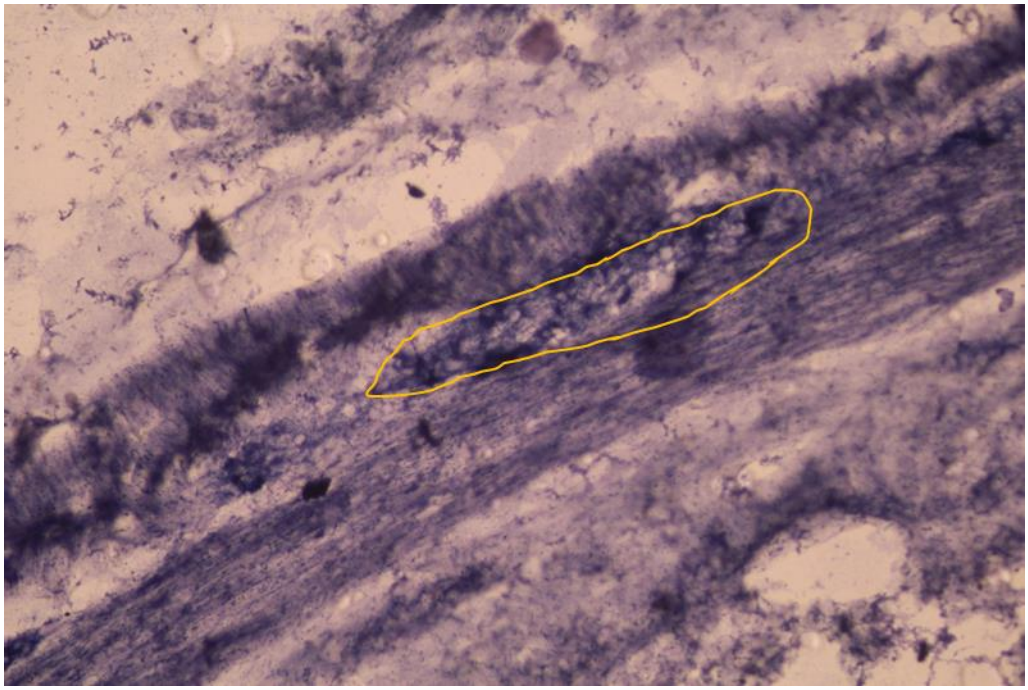


Fig 6: shows Spindle-shaped arrangements of the myenteric plexus.

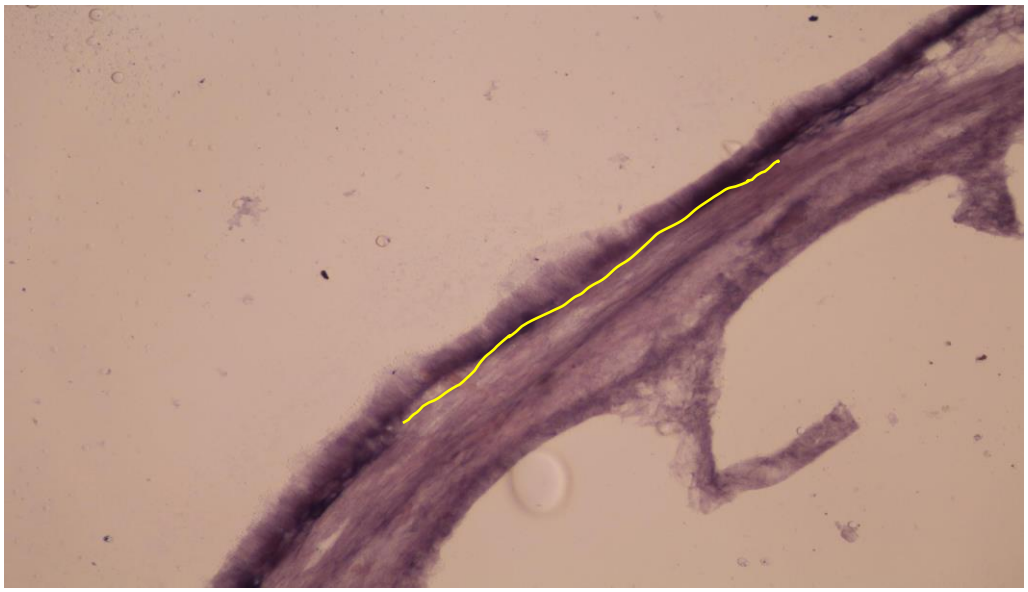


Fig 7: shows long inter ganglion distance (117.9µm) between the plexus.

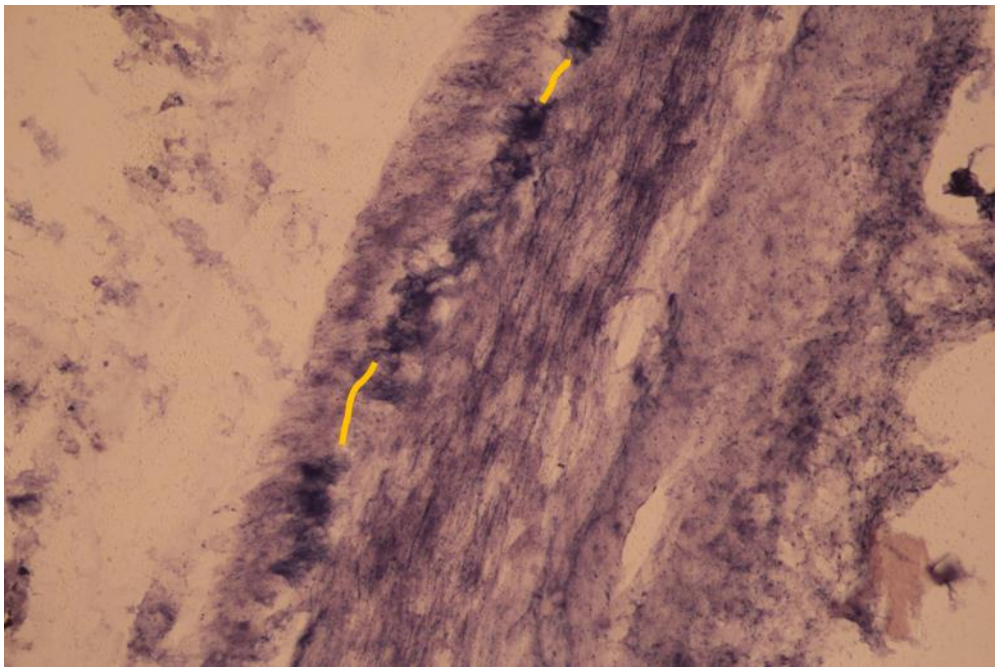


Fig 8: shows short inter ganglion Distance between plexus.

DISCUSSION

The quantitative morphometric measurements of enteric nervous system components are very useful as a diagnostic tool to identify disorders related to the innervation of the gut [12,15]. The present study aimed to measure the morphometric values of the entire large intestine.

Kim HK [12] et al studied changes in the structural components of the enteric nervous system in pediatric patients with intestinal pseudo-obstruction. The number, area and distribution of myenteric ganglia and the size, maturation, and number of ganglion cells in myenteric ganglia were identified. The mean inter ganglion distance in the hypoganglionosis group was greater than that in the control group. They suggested that the difference in results of statistical analysis of findings in the myenteric plexus depends on the location of the lesion. The comparative study with control sections suggested that location-matched reference values played an important role in diagnosing pathological changes in the myenteric plexus of the gut. Our study brought the normal values of all segments of the large intestine which further worked as location-matched reference values.

S. Singh et al [4] observed that the neuronal cell size increased gradually from 14 to 23 WG. Hitchcock et al [18] observed that the nerve cells and nerve density

peaked at 16 -20 weeks gestation and there was a fall in these values towards adult life. The present study reported that the number of nerve cells in a ganglion and the area occupied by nerve cells was increased up to 20 WG and these values were decreased more than 20 WG.

Hitchcock et al [18] observed in the human foetal oesophagus that the myenteric fraction peaked at 16-20 WG and the myenteric fraction fell during the late second trimester and became constant from 30 WG. The present study also reveals that these values were increased up to 20 WG and decreased above 20 WG.

Bhukya S et al [19] reported that the thickness of the circular muscle increased from 11WG to 19WG, but it was static until 26WG in the ascending and sigmoid colon. The thickness of the circular muscle in the oesophagus showed it increased continuously from 11 to 26 WG. The development of longitudinal muscle showed a pattern of continuous, uniform thickness increases in all segments from 13 to 26 WG. The circular muscle thickness was static up to 26 WG and longitudinal muscle thickness was slightly increasing up to 26 WG. The overall thickness was decreased when compared to the previous group of ≤ 19WG where both values were increasing. The present study revealed that the total muscular area increased up to 20 WG and decreased after 20 WG. It is statistically significant.

Bhattarai et al [9] studied the Histomorphology of enteric neurons and enteric ganglia in different layers of the human fetal colon. They reported that the distance between the enteric ganglia increased from 3.12 µm to 49.4±26.58µm in serosa and from 10.2 ± 6.29µm to 96.1±18.01µm in between the muscle layers whereas in submucosa it fluctuated as the gestational age progressed. In our study, the mean value of inter ganglion distance between the muscle layers ranges from 78.86 to 126.94 µm in the aborted foetus of less than 20 weeks of gestation and the mean value ranges from 66.41 to 107.67 µm in the aborted foetus of more than 20 weeks of gestation.

CONCLUSION

There are few studies on the quantification of the myenteric plexus in the colon of aborted foetuses. The normal values will help accurately diagnose and determine the pathological stage of the colon in gut motility disorders. The values were increased up to 20 weeks of gestation and were decreased in above the 20 weeks of gestation. The values were high in the middle part of colon and were decreasing on both sides. It implies a phenomena that they appear first in the middle part of the gut and later they were migrated towards caecum and anal canal.

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